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A new method for the selective quantitation of cyanogenic glycosides by membrane introduction mass spectrometry

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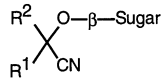
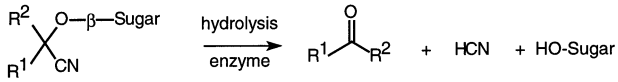
A new method is described for the rapid, sensitive, virtually interference-free, and selective quantitation of cyanogenic glycosides in aqueous extracts using membrane introduction mass spectrometry (MIMS). Selective monitoring, by either conventional MIMS or cryotrap-MIMS, not of HCN but of the co-released ketones (acetone and butan-2-one), when performed for both the crude cassava extracts and the linamarase–NaOH-hydrolyzed extracts, is found to offer an advantageous alternative to classic spectrophotometric methods based on HCN analysis for the selective quantitation of the two cyanogenic glycosides linamarin and lotaustralin expressed as both the free HCN content and the total cyanogenic potential (total HCN).

Introduction

Cyanogenic glycosides are naturally occurring toxins found in a large variety of plants and vegetables.[1] Owing to cyanogenesis, the release of the lethal HCN from cyanogenic glycosides or cyanolipids, the use of cyanogenic plants and vegetables as food sources may cause chronic or acute cyanide intoxication in animals and humans. All cyanogenic glycosides are derived from α-hydroxynitriles and have the general formula show below:

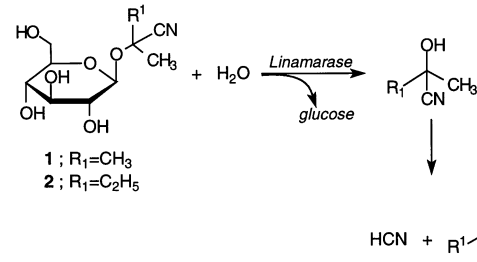


Hydrolysis or enzymatic action on the cyanogenic glycoside releases both a carbonyl compound and the lethal cyanide (eqn. (1)). The cyanogenic potential is, therefore, a crucial parameter that regulates the safe use of cyanogenic plants and vegetables as food.[1]



(1)

Cassava (*Manihot esculenta* Crantz) is a major cyanogenic vegetable rich in the two cyanogenic glycosides linamarin and lotaustralin at a near 97:3 concentration ratio, and is largely consumed in most tropical countries.[2–4] When cassava tissues are damaged or stressed, the cyanogenic glycosides are released and partially hydrolyzed by the endogenous enzyme linamarase (β-glycosidase) thus yielding both acetone and butan-2-one, and the lethal HCN (eqn. (2)). Also, the processing of cassava by the food industry when the roots are not peeled off generates large amounts of an aqueous extract called manipueira, which has an important carbohydrate composition but is richer in the potentially lethal cyanogenic glycosides. Hence, manipueira is used mainly as a soil fertilizer or as animal food. The toxicity of cyanogenic plants and vegetables and their extracts, such as *manipueira*, may result from both the free HCN (from the partial enzymatic hydrolysis) and the total cyanogenic potential, that is, the total content of cyanogenic glycosides.



(2)

Cyanogenic potential is most often measured by the indirect quantitation of the cyanogenic glycosides as the free and the hydrolysis-released HCN by classical spectrophotometric methods.[5–8] We now describe an alternative[9] and advantageous method that uses membrane introduction mass spectrometry (MIMS)[10] monitoring to selectively detect and quantitate the cyanogenic glycosides in aqueous extracts. MIMS is used to quantitate not HCN but the co-released carbonyl compound (eqn. (2)); when applied directly to cassava extracts, MIMS provides free HCN and total cyanogenic potentials (total HCN) comparable to those obtained by classic spectrophotometric methods of HCN quantitation. The MIMS method is advantageous because it is faster, virtually free of interferences (owing to the high and combined membrane and MS selectivity), and is able to quantitate, with high sensitivity, free HCN and total cyanogenic potential (total HCN) selectively for each of the two cassava cyanogenic glycosides.

Methods

A conventional MIMS system[11] was used for analysis of two crude cassava extracts: (a) the manipueira extract from the root containing the corky epiderm; and (b) the extract from the peeled root pulp (pulp extract). For diluted extracts, a cryotrap-MIMS system[12] was used (Fig. 1): the U-shaped trap tube was placed in liquid nitrogen for 15 min followed by its fast heating with the ballistic release of the condensed ketones to the mass spectrometer. The analyte solutions were pumped through the membrane interface at a rate of 3 mL min^{-1}, and an Extrel (Pittsburgh, PA, USA) pentaquadrupole mass spectrometer[13] fitted with high-transmission 3/4″ quadrupoles was used for ion detection. The membrane was an ultrathin sheet composite membrane of polyetherimide(polyester)–silicone with a 10 μm thick cross-linked silicone layer.[14] This ultrathin membrane displays much shorter response and recovery times and 2–3

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times better sensitivity for acetone and butan-2-one compared with conventional 250 μm silicone membranes.[13] To monitor both acetone and butan-2-one, selected ion monitoring (SIM) was applied for the corresponding molecular ions of *m/z* 58 and *m/z* 72 formed *via* 70 eV electron ionization (EI). For calibration, aqueous standard solutions of the two ketones were prepared by serial dilutions of stock 100 ppm solutions with doubly-distilled water.

To measure free HCN (as both the free acetone and free butan-2-one), 1 mL of the cassava extract was diluted in 11 mL of distilled water. To measure the total cyanogenic potential (total HCN), 4 mL of pH 6.0 phosphate buffer and 1 mL of an aqueous solution of linamarase (200 enzyme units) were added to 1 mL of the manipueira extract. The solution was then stirred for 15 min, 6 mL of 0.2 M NaOH aqueous solution was added, and followed by stirring for a further 5 min. Spectrophotometric quantitation of HCN was performed according to a reported procedure.[6] Three repeats were performed for each analytical sample.

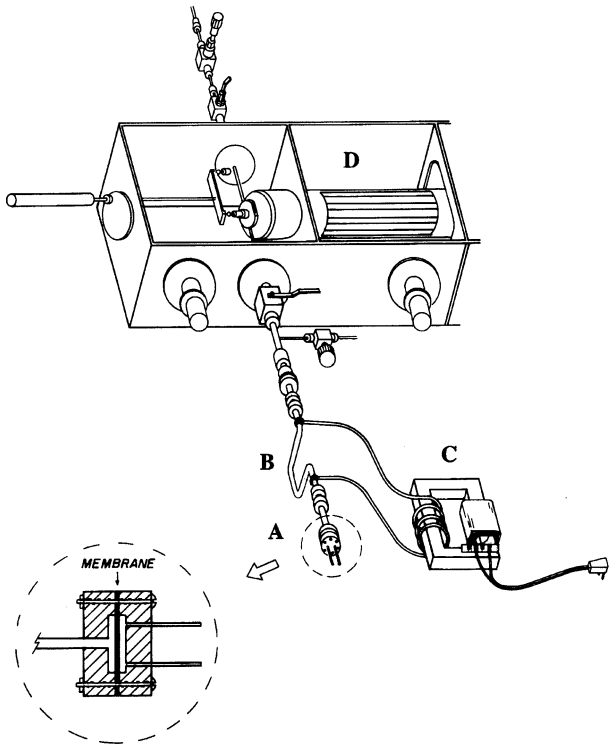


Fig. 1 A diagram of the CT-MIMS system used to indirectly quantitate (as both acetone and butan-2-one) the cyanogenic glycosides present in diluted cassava root extracts. (A) the membrane interface, (B) the U-shaped cryotrap tube, (C) the heating system, and (D) the mass spectrometer.

Results and discussion

Free HCN and total cyanogenic potential (total HCN)

Fig. 2 illustrates, for the detection of acetone in the manipueira extract, the typical profile observed for the analyte signal (SIM mode) in the various cycles of MIMS analysis (a similar profile was observed for butan-2-one). First, to obtain a calibration curve, standard aqueous solutions of acetone (Fig. 2) and butan-2-one were pumped through the system. Then, the manipueira extract diluted 12-times in water (to allow direct comparison with the hydrolyzed and also 12-times diluted extract; see the Methods section) was pumped through the system so as to measure its free HCN concentration as free acetone plus free butan-2-one. Then, the total cyanogenic potential was measured again as both total acetone and total butan-2-one (SIM of both *m/z* 58 and *m/z* 72) by pumping the linamarase–NaOH hydrolyzed manipueira extract.

From the data displayed in Fig. 2, a MIMS calibration curve for acetone was plotted (correlation coefficient of 0.998). The free and total concentration of the ketones, that is, acetone (Fig. 2) plus butan-2-one, measured for the crude and linamarase–NaOH hydrolyzed manipueira and pulp extracts, are directly related according to eqn. 2 and, after considering the 12-times dilution, to the amount of cyanogenic glycosides measured as both the free HCN and the total cyanogenic potential (Table 1). Comparable results were obtained when applying the classic spectrophotometric method of indirect HCN quantitation.[6]

Note that the free acetone concentrations measured in Fig. 2 are well above the detection limit (10 ppb) of acetone by MIMS. Note also that, when CT-MIMS is applied followed by fast heating and ballistic release of the analyte condensed in the U-trap (Fig. 1), a 100 times improvement in the detection limits for

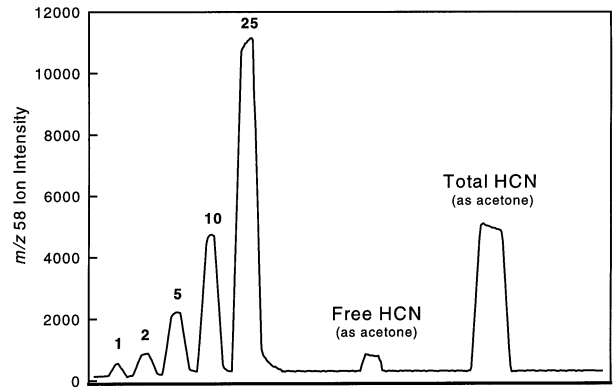


Fig. 2 Profile for the MIMS analysis using selected ion monitoring (*m/z* 58) of standard aqueous solutions of acetone and of both the crude manipueira extract (free HCN as acetone) and the linamarase–NaOH hydrolyzed extract (total HCN as acetone).

Table 1 Free HCN and total cyanogenic potential (total HCN) of cassava root extracts from 3 samples measured either as ketones (acetone plus butan-2-one) by MIMS or as HCN by a classical spectrophotometric method (SM)[6]

		Free HCN (ppm)				Total HCN[a] (ppm)			
			MIMS				MIMS		
Sample	Cassava extract	SM	Acetone	Butan-2-one	Total	SM	Acetone	Butan-2-one	Total
1	Manipueira	20	17	1.4	18	165	144	10	154
	Pulp	3.1	3.9	0.2	4.1	24	26	2.0	28
2	Manipueira	32	31	2.5	34	272	246	18	264
	Pulp	4.2	3.5	0.3	3.8	31	27	2.3	29
3	Manipueira	27	24	1.6	26	237	207	16	223
	Pulp	4.5	2.9	0.2	3.1	28	27	2.1	29

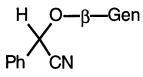
[a] Repeatability in total HCN for 3 repeats was near 6%.

acetone and butan-2-one (100 ppt) are achieved.[12] Hence, quantitation of linamarin as acetone and lotaustralin as butan-2-one (detection limits of 5 ppb by MIMS[11a] and 50 ppt by CT-MIMS[12]), can be performed with high sensitivity by both conventional MIMS and particularly by CT-MIMS for more diluted extracts.

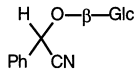
Conclusion

The quantitation of the co-released carbonyl compound by conventional MIMS or CT-MIMS offers an advantageous alternative to classic spectrophotometric methods based on HCN quantitation for fast, sensitive, virtually interference-free, and selective quantitation of free HCN and total cyanogenic potential (total HCN) in crude or diluted aqueous extracts of cassava, and in extracts from most cyanogenic plants and vegetables.

The present work has demonstrated the applicability of the MIMS method for cassava root extracts, but similar procedures should also be applicable to measure total or selective (or both) cyanogenic potentials of other cyanogenic glycosides; for instance, to quantitate (as benzaldehyde) amygdalin and prunasin (see structures below) found in apricot kernels.[1] The



Amygdalin



Prunasin

MIMS method, which combines membrane chemical selectivity with MS selectivity, should be particularly advantageous for extracts containing interferences known to respond to the spectrophotometric method.

Acknowledgements

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